MICROORGANISMS AND THEIR RELATION TO FEVER[1].

(PRELIMINARY COMMUNICATION.)

BY E. C. HORT
AND W. J. PENFOLD.

(*From the Lister Institute, London.*)

(With 27 Charts.)

IT appears to be widely believed that the fever of infective disease is due to absorption of pyrogenetic substances liberated from dead microorganisms by a combined process of lysis and extraction in the body of the infected subject. The diffusion of products of the action of bacterial autolytic enzymes on microorganisms after their destruction by the defensive agencies of the body is a variant on the same theme. Closely allied are the theories of protein fever, and of anaphylactic fever, in so far as they relate to the supposed possession by microorganisms, or their split products, of pyrogenetic properties. The absorption of alien protein, or of derivatives of alien protoplasm, from organisms that are dead, is, in short, the essential feature of this conception of fever production.

At present there is no satisfactory evidence available to support this theory. On the contrary, there are many clinical facts in the way of its acceptance, though their interpretation is a matter of great difficulty.

In 1909 one of us therefore suggested(1) as an alternative view that the source of fever in infection may be, not the dead cells of the invader, but the tissues or fluids of the host. In other words it is possible that fever may be an index, in infective disease, not of the death and lysis of causal organisms, but of the ability of the living organism, or, if it be

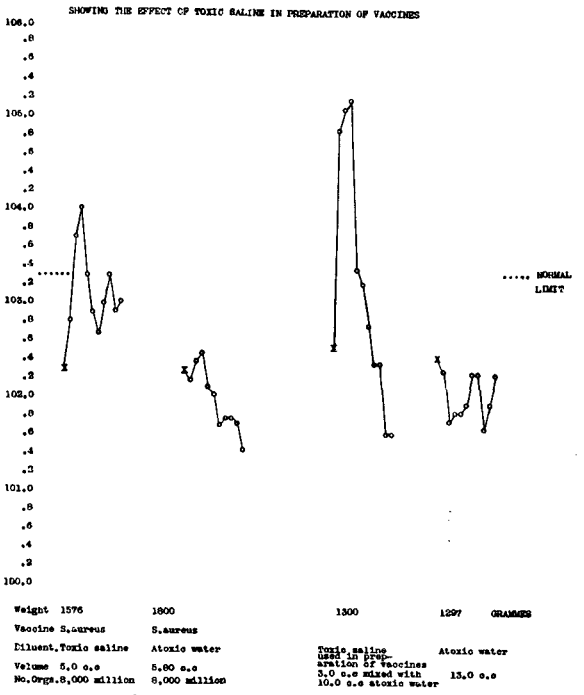
[1] The experimental expenses of this research were defrayed by the Constance Research Fund administered by one of us (E. C. H.). The authors wish to record their thanks to the Council and Directors of the Lister Institute for the laboratory facilities placed at their disposal.

damaged, of its derivatives, perhaps enzymic in nature, to liberate from the natural medium on which it grows pyrogenetic bodies that it does not itself possess. This might be brought about in one of two ways. The pyrogen might be a product of the action of autolytic enzymes of the cells of the host on the substrate provided by these cells after their destruction by the toxic agents of microorganisms, or it might be a product of the action of bacterial enzymes on the same substrate. In either case the essential factor in the production of fever would be the liberation of pyrogenetic substances of native origin, as opposed to the alien source of supply suggested by the generally accepted view.

Thus broadly stated these two theories as to the source of pyrogen in infective disease are mutually antagonistic. In order to make clear the points we wish to establish it is necessary to refer to certain fallacies in all experimental enquiry into the fever produced by microorganisms.

(1) We have recently shown that the injection into animals of various substances suspended or dissolved in saline or water may give rise to fever which depends, not on the nature of the substance injected, but on contamination of the diluent employed. Water or saline may be absolutely free from living or dead microorganisms at the time of injection, and may yet be strongly pyrogenetic. The contaminating principle in such water or saline we have shown (2) to be a heat stable body of small molecule, incapable of removal by ordinary methods of filtration, and bearing no relation to the degree of demonstrable infection of these liquids. This pyrogenetic substance may or may not be a derivative of bacterial cells, but whatever its nature its unsuspected presence has given rise to belief in many different forms of fictitious injection fever, such, for example, as water fever, salt fever, sugar fever, ferment fever, tissue fever, salvarsan fever and so forth. In the study of bacterial fever the same fallacy that we demonstrated in the forms referred to has crept in, and we find that many organisms of different types owe their apparent pyrogenetic properties to the fact that the saline used for purposes of suspension or solution has become contaminated with the body described. The cognate study of protein fever is, we now find, materially affected by the same unsuspected fallacy, which therefore directly affects the question of bacterial protein fever. And this is particularly the case in anaphylactic fever where multiple injections of bacterial or other protein suspended in water or saline must of necessity be given. Our charts will show that if precaution be taken to use water that has been freshly distilled the apparent pyrogenetic properties of certain organisms at once disappear. This is illustrated by Chart 1.

In the cases injected we have also been unable to obtain any evidence of anaphylactic fever. Our experiments on this subject will be dealt with in a separate paper. To what extent, however, our results affect the value of the evidence offered by Vaughan(2a), Wheeler(2b), Friedberger(2c), Schittenhelm and Weichardt(2d), and others on the subject of bacterial fever, endotoxin fever, protein fever and anaphylactic fever may



Interval between observations 30 minutes.

Chart 1.

be seen by reference to a paper recently read by us[1]. If this be studied in the light of the knowledge we now possess it will be realized how manifold are the chances of error introduced into all experiments of this nature involving the use of water or of saline of unknown origin. It is necessary to remember that demonstration of the sterility of these liquids by attempts at cultivation affords no guarantee of the absence of the heat stable pyrogen described.

(2) There is a second source of error not yet fully recognized. This was introduced by Friedberger(3) when he selected the guinea-pig for his

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experiments on anaphylactic fever. In this animal as Hort showed(4) in 1910 the temperature is so unstable, partly owing to the impossibility of avoiding injury to the rectal mucous membrane when taking thermometric observations, that it is impossible to draw safe conclusions as to temperature changes after injection. Chart 2 demonstrates the accuracy of this statement, as the temperature curve given is that of an animal which received no injection. In order to show that this was no chance observation records were taken for several days of six other animals. In all cases the same kind of results was obtained. In the rabbit fortunately this objection does not hold as our numerous controls will show.

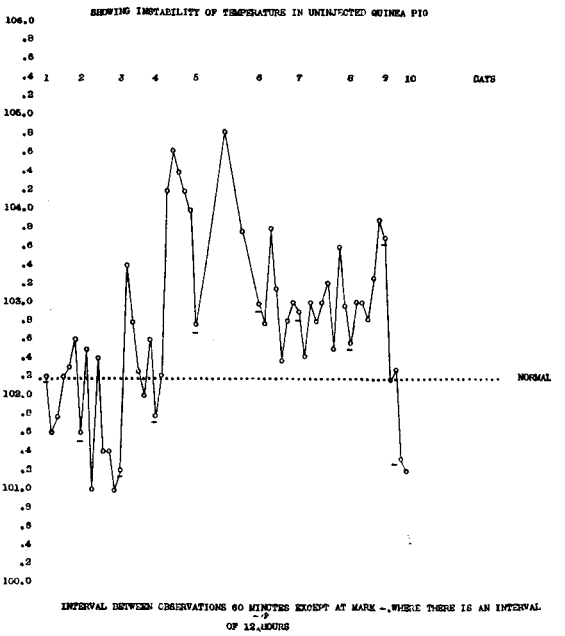

Chart 2.

(3) In spite of care taken to avoid these two sources of error we soon encountered a third of a totally unsuspected nature. We find that in determining the pyrogenetic properties of small fractions of a broth culture of *B. typhosus*, for example, increase of the volume of the diluent often has a striking effect in increasing the extent of fever produced. This is shown in Charts 3, 5 and 7. Moreover if a larger fraction of the culture, or even the same fraction, which when given alone is non-lethal, be injected in a volume of water equivalent to from

about 1/100th to 1/25th of the body weight death often ensues in an hour or two. This is shown in Charts 3, 6, 7 and 8. In Chart 9 are given control observations showing the absence of lethal effect in the case of undiluted cultures. Control observations as demonstrated in Chart 4, show that neither fever or death is ever produced by pure water alone after injection in these quantities. The former of these effects is the only one we can pursue here. In all these cases the cultures

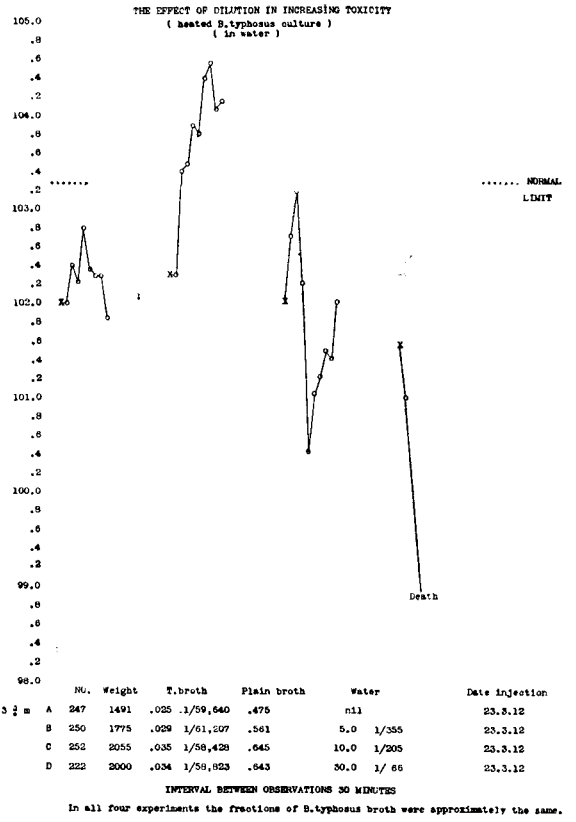

Chart 3.

were injected 18 hours after inoculation of the broth. The charts show the importance of recognizing this source of error as regards the interpretation of fever. At first sight it would appear that an explanation of this might be found in the facility afforded to *in vitro* extraction by the addition to a constant weight of bacterial culture of increasing

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quantities of distilled water. We find, however, that the same phenomenon may occur when normal saline is used instead of water. We also find that if water or saline be injected for some minutes before the fractions of culture are injected this greatly intensifies the effect, though the exact time limits of this curious form of sensitisation by

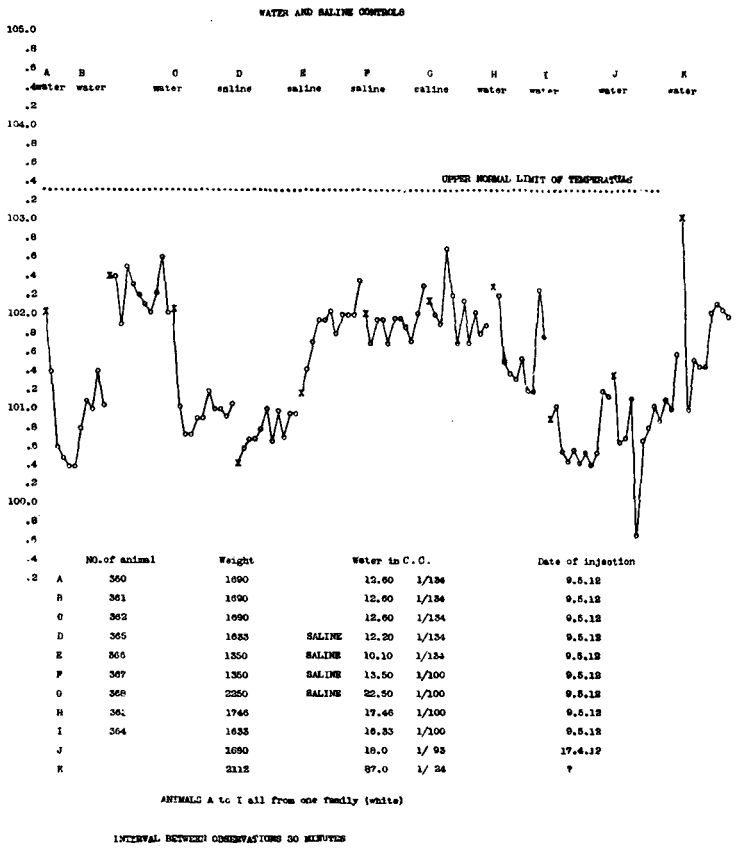

Chart 4.

water or saline have not yet been determined. This is shown in Charts 5, 6 and 7 as regards water. Clearly therefore the increase of toxicity induced by increase in the volume of diluent cannot be merely due to increased facility of extraction of toxic bodies in the test tube. We attempt no explanation, but are content for the moment to record the facts.

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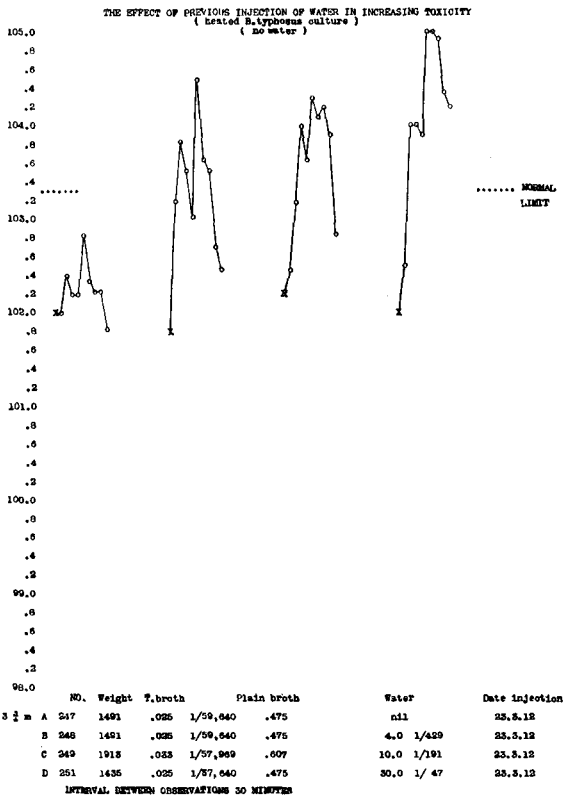

Chart 5.

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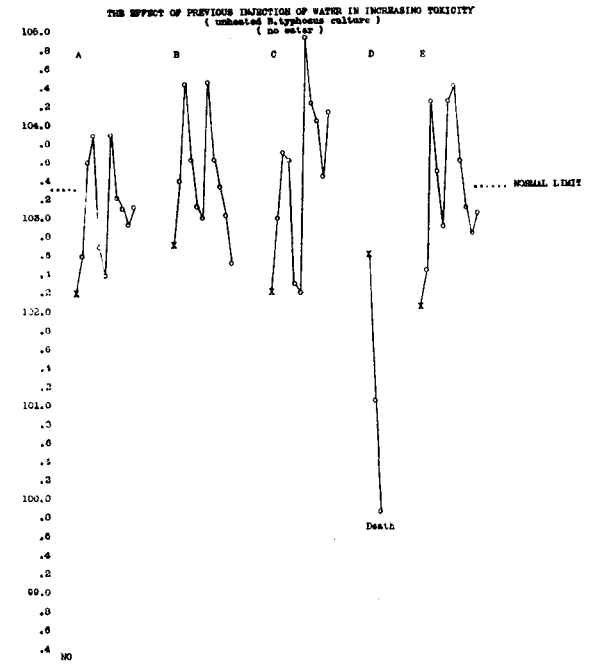

Chart 6.

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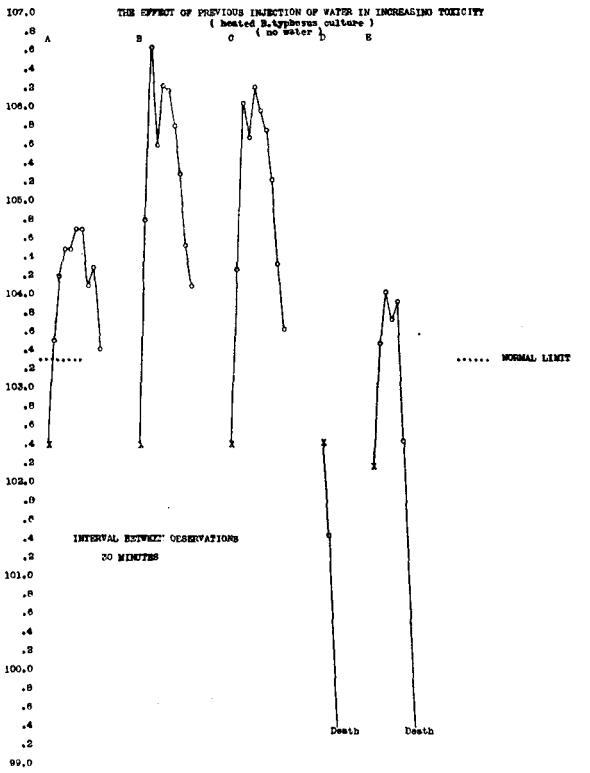

	NO.	Weight	T.broth		Plain broth	Water		Date injection
4 m A	270	1690	.027	1/62,592	.133	nil		16.4.12
B	271	1690	.027	1/62,592	.133	4.20	1/402	16.4.12
C	272	1913	.032	1/59,781	.146	9.50	1/201	16.4.12
D	722	1576	.024	1/65,666	.126	23.60	1/ 66	16.4.12
E	731	1941	.030	1/64,700	.160	48.50	1/ 40	16.4.12

Chart 7.

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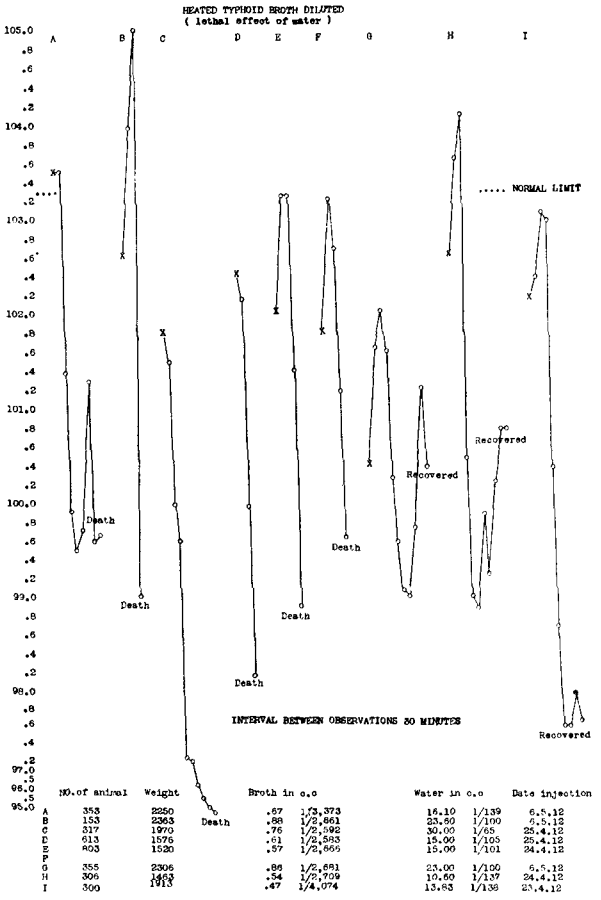

Chart 8.

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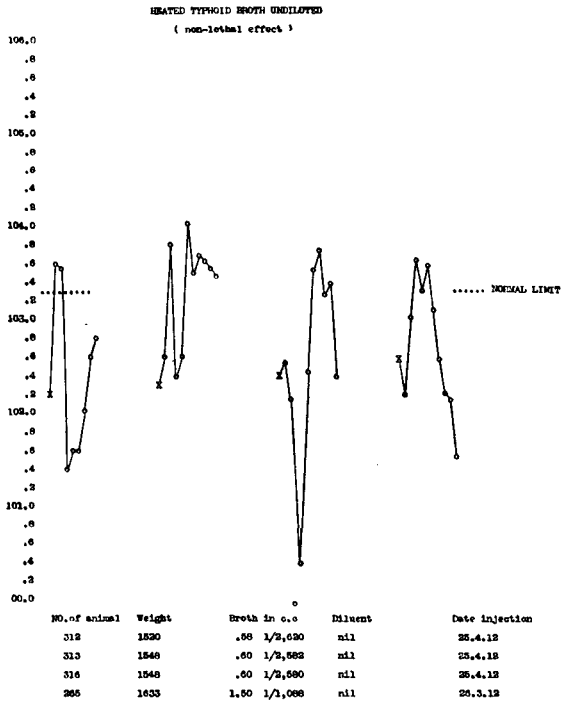

Chart 9.

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(4) There is another source of fallacy in connection with the diluent employed which is equally difficult to explain. If a suitable quantity of a heated or unheated culture of *B. typhosus* be injected, after suspension, in constant volumes, of distilled water, relatively to body weight, fever appears in all the animals injected. The extent of fever may vary in individual animals to a slight extent, but the reaction never fails.

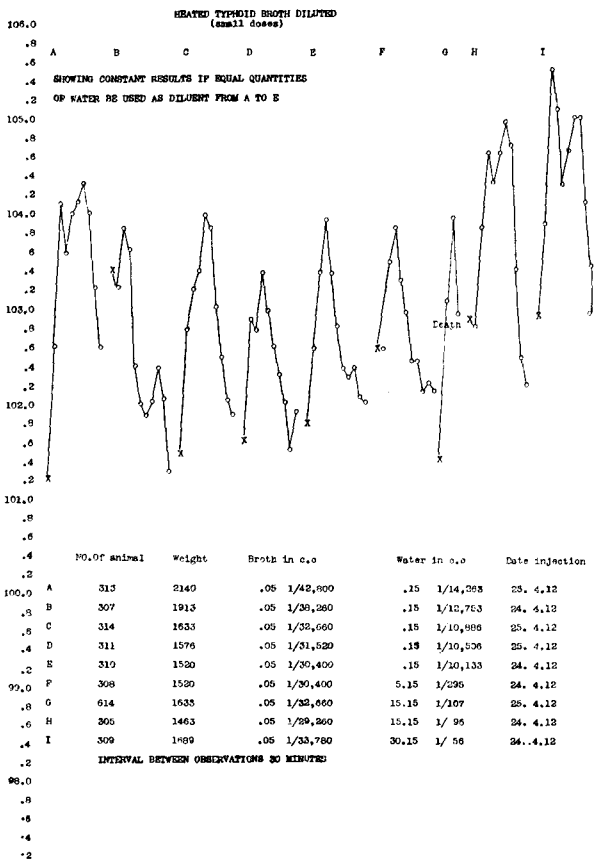

Chart 10.

We make this statement after injection of nearly two hundred animals with this organism. Examples are shown in Chart 10. On the other hand we find that if normal saline, ·85 %, be injected in the place of the water most inconstant results follow in regard to fever. In a pair of animals for example injected with the same fraction of culture and the same volume of saline, relatively to body weight, one animal may show

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a rise of temperature to 105·0° F., whilst the other animal is scarcely affected. This is shown in Chart 11. This phenomenon we frequently noticed in our work on fictitious salt fever published last year. We noticed the same thing also 12 months ago when studying the relative toxicities of different kinds of toxic exudates dissolved in water or in saline. Recognition therefore of this source of fallacy is certainly

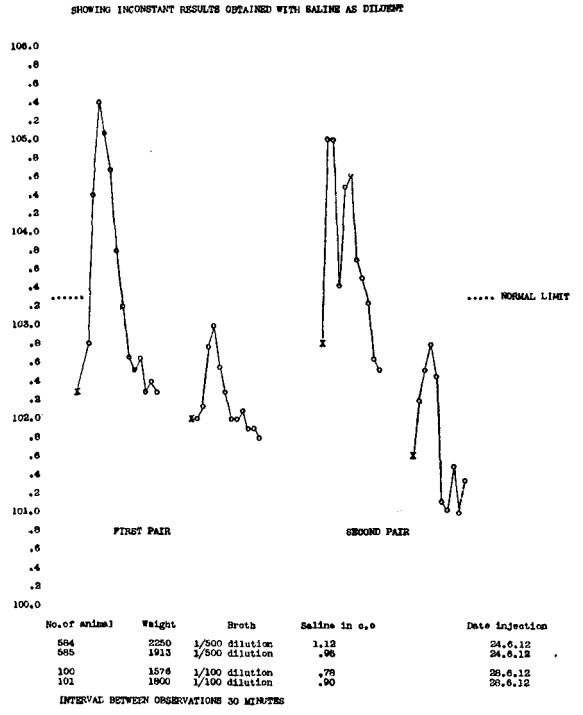
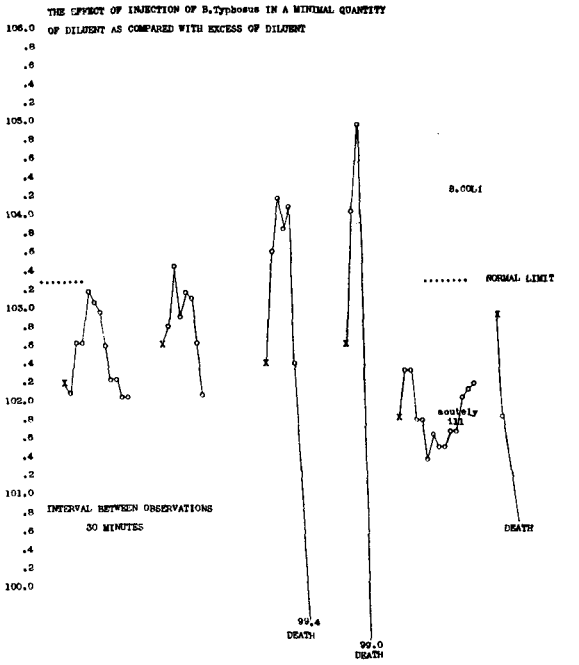


Chart 11.

necessary in studying experimental fever, and other indications of toxic action. In all the experiments here quoted distilled water that we have prepared each day for the purpose has been used as the diluent, unless otherwise stated.

(5) The last source of error we wish to refer to in connection with bacterial fever investigation is due to the fact that the effect may be masked if the dose given be too large. If a small but sufficient fraction of a *B. typhosus* culture be injected a fairly definite extent of fever is always produced if water be the diluent, as exemplified in Chart 10. If, however, a larger quantity of both culture and water be given a

greater amount of fever ensues, as already described, up to a certain point. If this point be passed, as was seen in Chart 8, a large injection produces first a rise of temperature and then a fall, often of six or more degrees before death takes place. Recovery, however, may occur. The fall noted is either due to an excessive dose of the pyrogen producing an inverted effect, as has been often suggested though never proved, or it is



NO. of animal	Weight	Approx. number of organisms		Water	Date injection
200	2335	300 million	heated	.05	26.3.12
201	1690	300 million	unheated	.05	17.4.12
731	1941	4 million	heated	48.50	24.4.12
153	2363	134 million	heated	23.60	6.5.12
OBSERVATIONS AT INTERVALS OF 30 MINUTES					
1	1800	30 million	unheated	1.0	1.7.12
2	1800	30 million	unheated	1.0	1.7.12

Chart 12.

due to the action of an excess of endotoxin or other depressant body. Owing to difficulties to which we shall later refer it is not possible at present to say if a small dose of typhoid toxin produces a fall or rise of temperature, the main difficulty being caused by inability to obtain an endotoxin uncontaminated by products of the artificial medium on which

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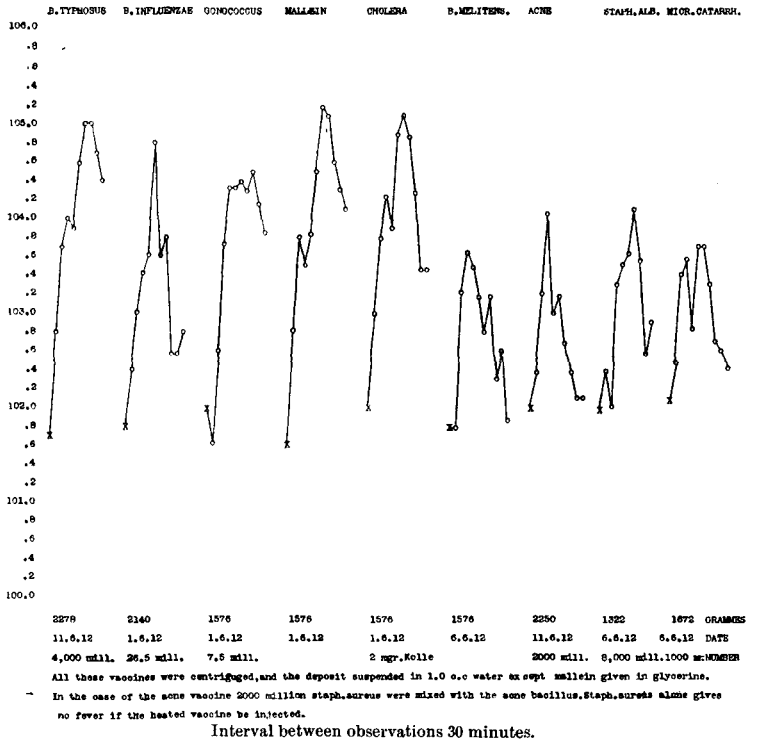
the organism has been grown. The rise of temperature followed by fall so often seen after an injection of a large dose may be due to excess of one body or to the resultant action of a couple of which each member is pulling in opposite directions. We do not forget the depressant effects of nitrites and ptomaines from the broth. The whole question requires much further study, and cannot be pursued here. There is, however, a further fact which must be mentioned. If a massive dose of

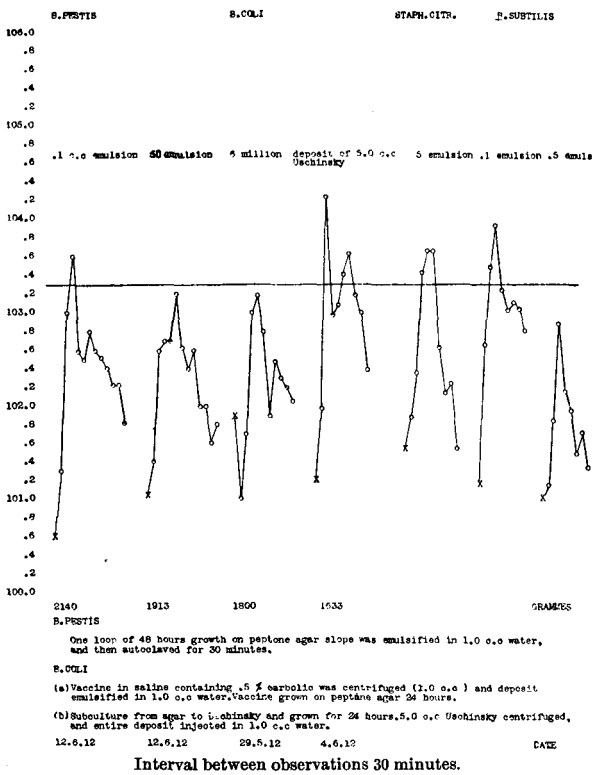


Interval between observations 30 minutes.

Chart 13.

culture far beyond the lethal point be injected it sometimes happens that no disturbance of temperature is produced, though the animal may become acutely ill. This is shown in the last two experiments in Chart 12 where a culture of *B. coli* has been injected. Absence of temperature reaction after injection in distilled water of small doses above the vanishing point on the other hand we have never seen. Finally we have on several occasions noticed that if a large number of living or dead *B. typhosus* organisms deprived of all removeable

traces of medium be injected in as dry a state as possible short of absolute desiccation there is not only no marked disturbance of temperature, but the animal appears to be entirely unaffected either at the time of injection or later. This is well seen in Chart 12 in the first two experiments shown. The second pair of experiments shows how the increase of diluent lowers the lethal point.

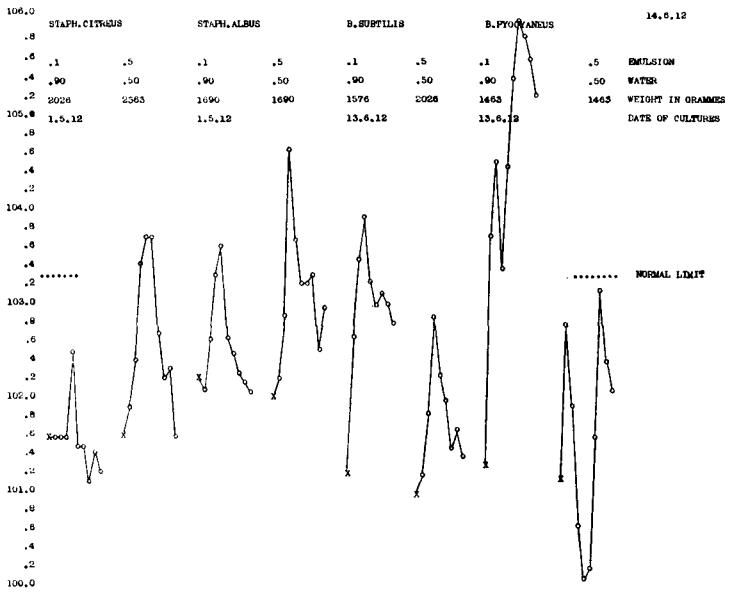


Interval between observations 30 minutes.

Chart 14.

Bearing all these possible sources of error in mind we then examined the pyrogenetic properties of many different kinds of organisms. We find that we are able to divide the organisms we examined into two groups, according to whether they do or do not after death by heat excite fever when injected into healthy rabbits. We selected heat as our method of destruction because we wished to avoid the introduction of any extraneous poison, and because, as we had already shown, the pyrogen of distilled water was heat stable. In all cases unless otherwise

stated, we submitted the cultures, or the centrifuged organisms, to heating for two minutes in a water bath brought to, and maintained at, the boiling point. We soon found that some varieties of organisms produce immediate fever whether injected alive or dead. It became necessary therefore, when studying the immediate and remote effect of dead organisms, to be absolutely certain that complete sterilization had taken place. The use of filters in the study of injection fever is quite inadmissible, as we have elsewhere shown, owing to the difficulty of obtaining



For each emulsion a loop of growth was taken up in 1.0 c.c of water and divided as stated.
Each emulsion was boiled for 5 minutes, except in the case of B. subtilis which was autoclaved for 30 minutes.
Interval between observations 30 minutes.

Chart 15.

filtrates free from inorganic impurities. In group A will be found the organisms that we have found to produce fever on intravenous injection, after excluding the fallacy arising from injection of toxic saline or water. They are *B. typhosus, B. coli, B. pyocyaneus, B. subtilis, B. melitensis, B. pestis, Micrococcus catarrhalis, S. albus* and *citreus, B. influenzae, Gonococcus, B. acne,* the cholera vibrio and also mallein. Examples are shown in Charts 13, 14 and 15. Group B on the other hand contains organisms that we have found to produce when injected after destruction by heat no fever whatever. They are *Staphylococcus*

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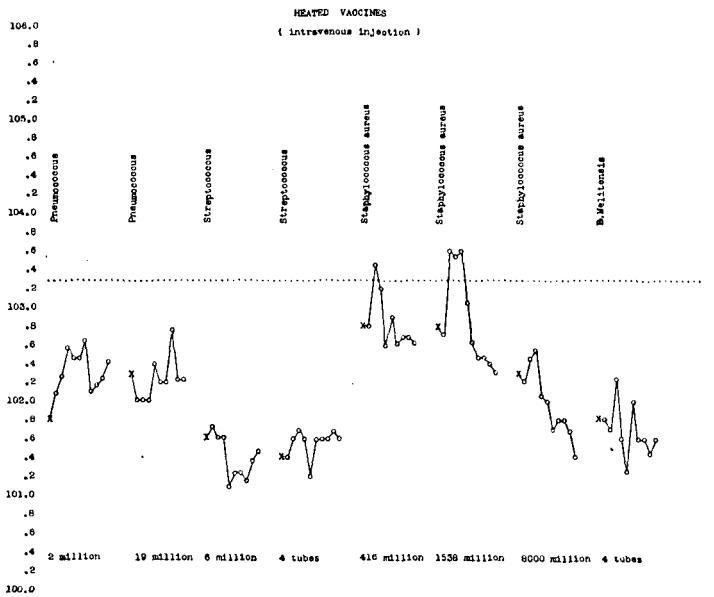
aureus, *Pneumococcus*, *Streptococcus*, *B. diphtheriae*, *B. anthracis*, *B. tetanus*, tubercle bacilli and also T. R. tuberculin. Examples are shown in Chart 16. In Charts 17, 18, 19 and 20 are shown the results of experiment with rising quantities of organisms in the case of many different members in group B. In no single case have we observed fever. In the case of *B. typhosus* we examined five different strains, in the case of *S. aureus* eight strains. In the case of the other organisms in groups A and B only single strains were as a rule examined. We



Interval between observations 30 minutes.

Chart 16.

feel therefore that the results obtained in the case of *B. typhosus* and *S. aureus* are likely to hold good for all strains of these organisms grown on artificial media. To what extent this will be found to apply to organisms where only one strain was examined can only be determined by further experiment.

We find that the nature of the medium on which an organism such as *B. typhosus* is grown affects the pyrogenetic properties of this organism. Less fever is produced for example by growth on agar than by growth on peptone broth. We have elsewhere shown that heated organisms collected from agar plates after exposure to aerial

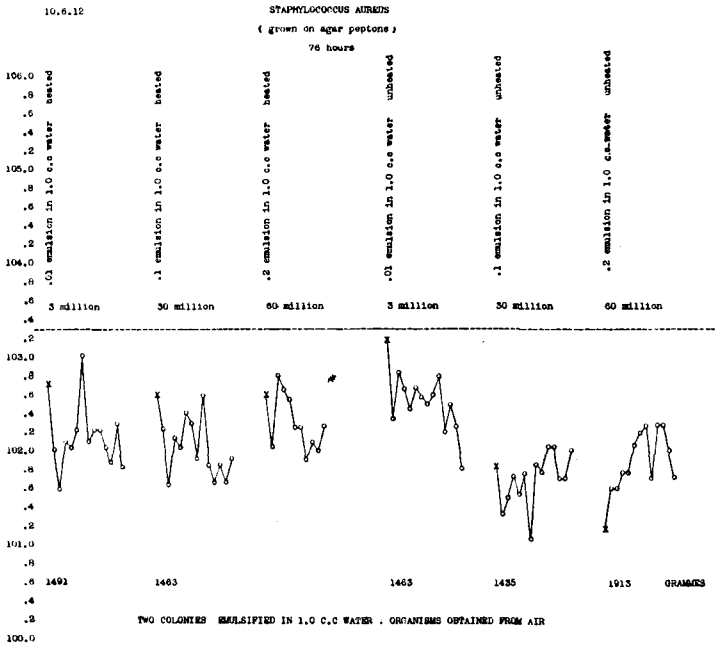
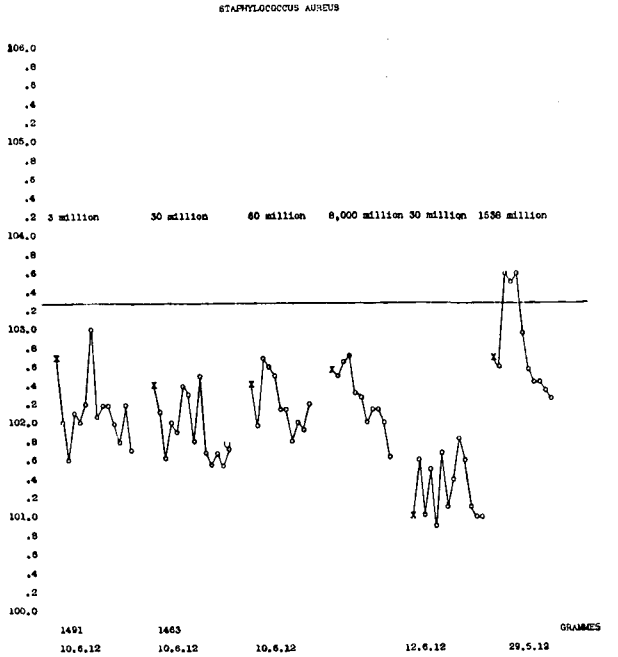


Chart 17.



Interval between observations 30 minutes.

Chart 18.

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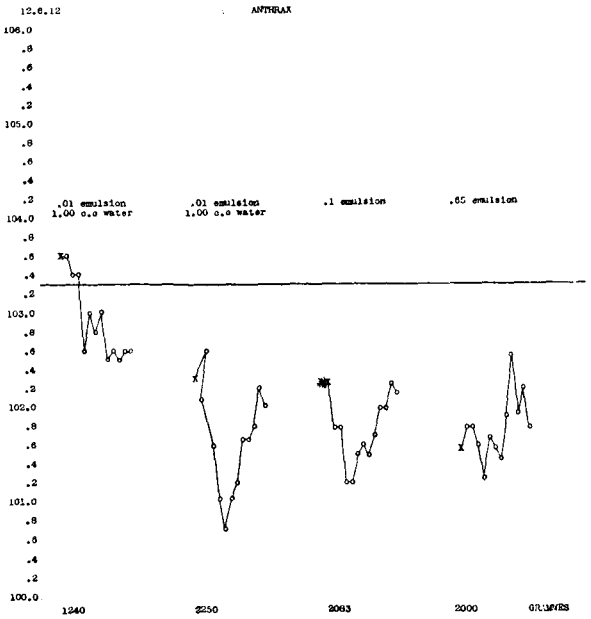
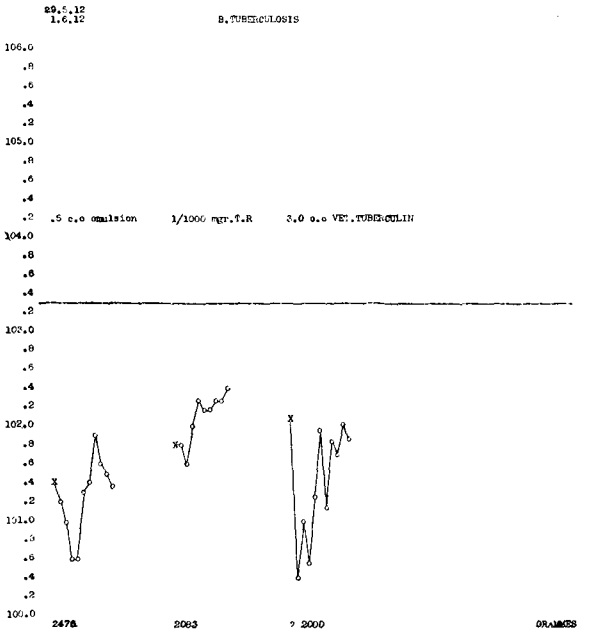


One loop of 24 hours growth on peptone agar slope was emulsified in 1.0 c.c water. and then autoclaved for 30 minutes.

Interval between observations 30 minutes.

Chart 19.



In first experiment a loop of heated peptone agar culture, date 24.1.12, was emulsified in .50 c.c water and injected in 5.0 c.c water.
In second experiment tuberculin was injected in 1.20 c.c water.
In third experiment veterinary tuberculin was injected in glycerine subcutaneously

Interval between observations 30 minutes.

Chart 20.

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infection may produce fever, whilst after similar treatment the same varieties of heated organisms grown in water or saline will produce none. We have also shown that this is not merely a question of numbers. We hoped that growth of *B. typhosus* or *B. coli* in non-protein media, such as those of Capaldi, or Uchinsky, might be of service in helping us to determine the source of the pyrogen in the case of organisms in group A. We found, however, that we could not obtain growth on these media of *B. typhosus*, whilst with *B. coli* that gave

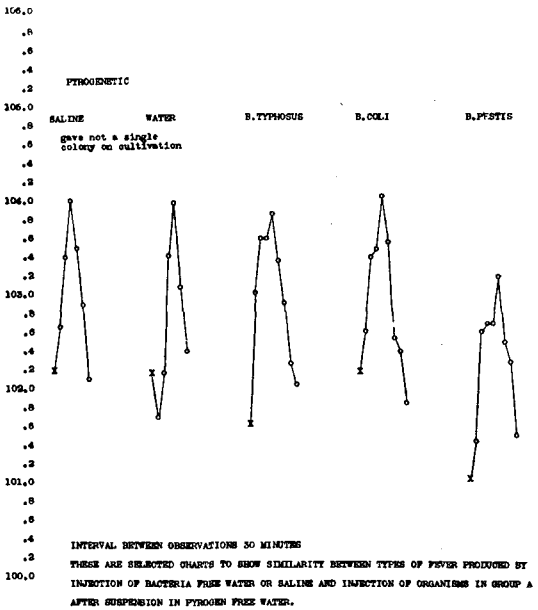

Chart 21.

luxuriant growths we could never be sure that we had not carried over pyrogen derived from the broth. We find that a culture several weeks old gives rise to less fever than does a culture of 24–48 hours' growth. The type of fever produced by injections in distilled water of cultures of *B. typhosus*, an organism which illustrates very well the fever produced by other organisms in group A, after destruction by heat, is strikingly similar in every respect to the fever produced by injections of bacteria-free water or saline containing the heat stable pyrogen we have described. This is shown in Chart 21. The fever begins as a rule within a few minutes of injection, unless

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too large a quantity be given, attains its maximum within 2 to 4 hours, according to the dose, and returns to normal within 5–6 hours. There is a sharp rise, a sharp fall, a short duration and no latent period. Exactly the same type of fever is produced by the injection of living cultures of *B. typhosus*, rising and falling at the same time within very close limits. Whether live or dead organisms be injected the height and duration of the fever run closely parallel if equal quantities of culture and water, relatively to body weight, are given, provided that

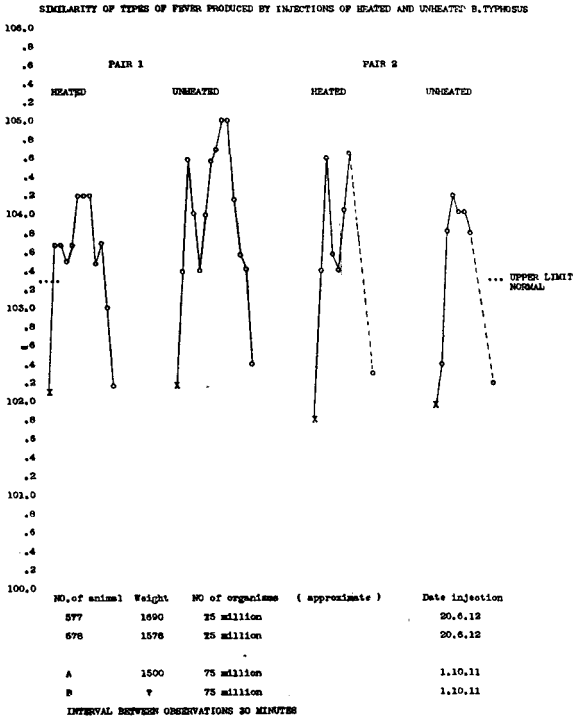


Chart 22.

the doses are small. This is shown in Chart 22. The type of fever curve obtained by injection of living or dead cultures in group A is quite unlike any known form of clinical fever occurring under natural conditions of infection in man or animals. We wish to emphasize the fact that though the pyrogen injected in the case of the heated culture is heat stable the fever induced by injection of the living culture lasts no longer, and does not pass into a continuous fever, as one would expect it

to do if a constant supply of dead organisms was being kept up owing to multiplication, and subsequent partial destruction, in the body. The pyrogen, in short, introduced with the living organism is also heat stable. The significance of this point we shall again refer to. Repeated observation for several days after injection reveals no secondary rise of temperature whether the organisms be injected alive or dead. This is shown in Chart 23. The inability of living *B. typhosus* to produce a late continuous fever in the rabbit is possibly associated with the difficulty of

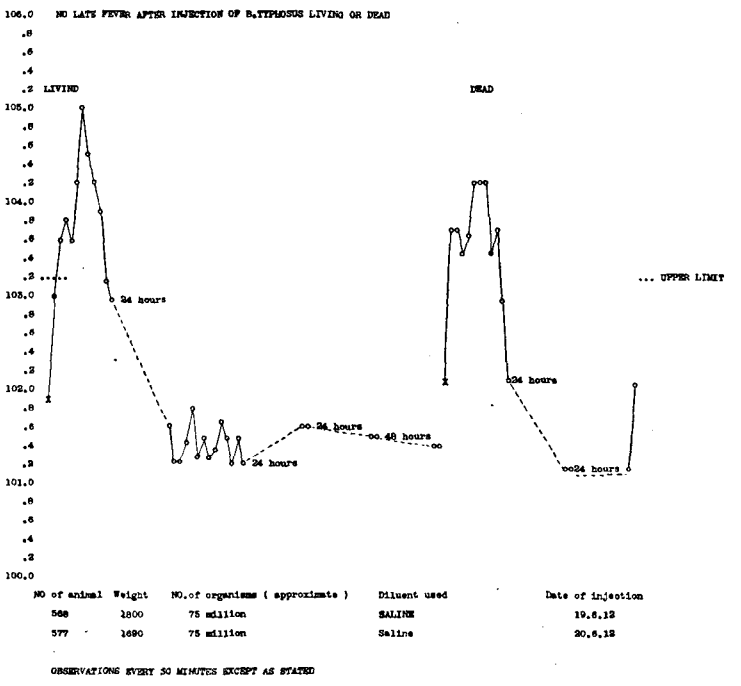
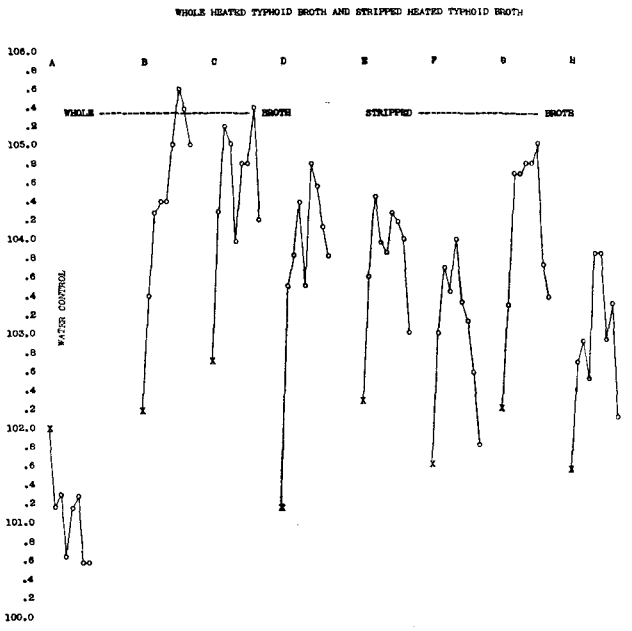


Chart 23.

producing a continuous infection in this animal with this organism. This point is of particular interest in connection with the well-known power of the rabbit to act as a carrier of *B. typhosus* without the induction of symptoms of intoxication. We have found it quite impossible to produce any sign of immunity to the injection of the pyrogen of *B. typhosus*. We find it therefore difficult to believe that the typhoid carrying rabbit does not suffer from fever in consequence of being provided with an antipyrogen.

In order to differentiate between the immediate fever and the late fever (if any) produced by injection of living organisms in group A it is necessary to select a type that will give a continuous infection. This we are now studying in the case of *B. coli*. So far we have been unable to produce any late fever with *B. coli*, whether injected alive or dead. The next fact we wish to present in connection with *B. typhosus* is that



	NO.	Weight	B.T per kilo	Actual no.	Water	Date injection	Whole broth	Stripped broth
A	426	1576	nil	nil	7.80 1/202	24.5.12	nil	nil
B	418	1576	.8 m	1.5 m	7.80 1/202	24.5.12	.1 1 in 10	nil
C	420	1576	.8 m	1.5 m	7.80 1/202	24.5.12	.1 1 in 10	nil
D	421	1576	.8 m	1.5 m	7.80 1/202	24.5.12	.1 1 in 10	nil
E	422	1576	nil	nil	7.80 1/202	24.5.12	.nil	.1 1 in 10
F	424	1576	nil	nil	7.80 1/202	24.5.12	nil	.1 1 in 10
G	423	1576	nil	nil	7.80 1/202	24.5.12	nil	.1 1 in 10
H	425	1576	nil	nil	7.80 1/202	24.5.12	nil	.1 1 in 10

INTERVAL BETWEEN OBSERVATIONS 30 MINUTES

Chart 24.

whether we inject in suitable quantity, the culture medium plus organisms, washed organisms separated from the medium by the use of the centrifuge, or the supernatant layers of the broth from which the organisms have been thrown down, the result is always the same—fever. This is shown in Charts 24 and 25. Control observations on the apyrogenetic effect of uninfected broth are seen in Chart 26.

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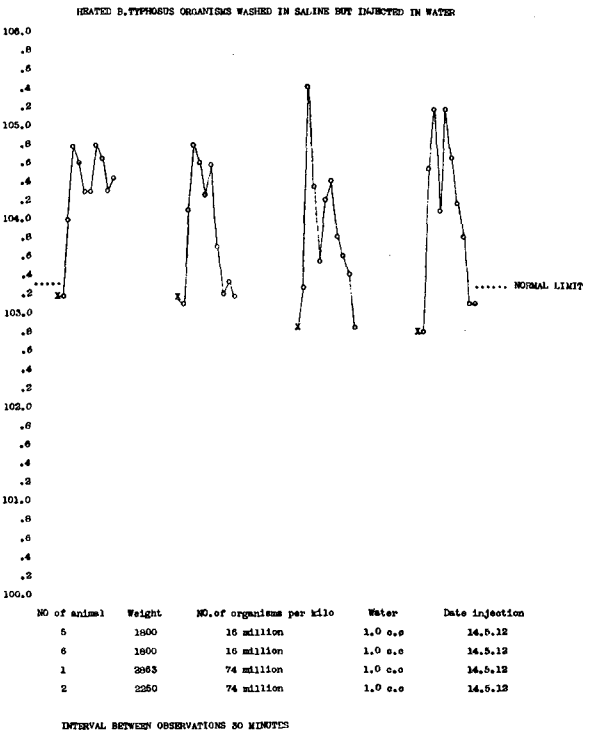
Moreover whether the organisms, the suspension of organisms in the broth, or in the broth deprived of its organisms, be submitted to heat or not, fever always follows if a correct dose be given. The pyrogen of each fraction is heat stable, and the fever produced in each case is of precisely the same type. The comparatively narrow limits of temperature reaction in response to the injection of pyrogen in the rabbit makes it difficult to determine if the injection of broth plus organisms gives rise to more fever than does the injection of organisms alone or of broth



NO of animal	Weight	NO. of organisms per kilo	Water	Date injection
5	1800	16 million	1.0 c.c	14.5.12
6	1800	16 million	1.0 c.c	14.5.12
1	2363	74 million	1.0 c.c	14.5.12
2	2250	74 million	1.0 c.c	14.5.12

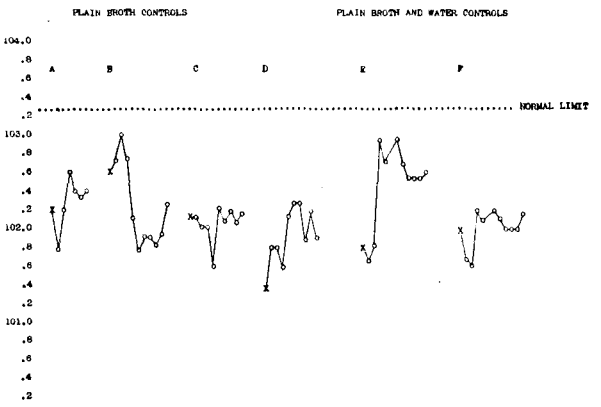
INTERVAL BETWEEN OBSERVATIONS 30 MINUTES

Chart 25.

alone. The fact, however, that after prolonged heating to 120° C. the culture does not produce a greater effect in terms of fever than is seen after injection of the living organism affects to some extent the question of the share taken by derivatives of dead bacteria in fever production. We further find that if *B. typhosus* organisms are separated by the centrifuge from the broth in which they are grown, or are collected from agar, the pyrogen cannot be wholly removed by prolonged washing with

water or with fresh uninoculated broth. The washings are, however, strongly pyrogenetic. If on the other hand the precipitate is washed with hydrogen peroxide, which is subsequently centrifuged or driven off by heating in the water bath, injection no longer produces fever. The same destruction of pyrogen is produced if the precipitate of organisms be freed from all excess of liquid by the aid of a Pasteur pipette, and subsequently allowed to stand for 12 hours in the cold.

After examination of numerous films we find that a precipitate of *B. typhosus* not treated in either of these ways does not stain nearly so well with methylene blue as it does if either of these methods have been



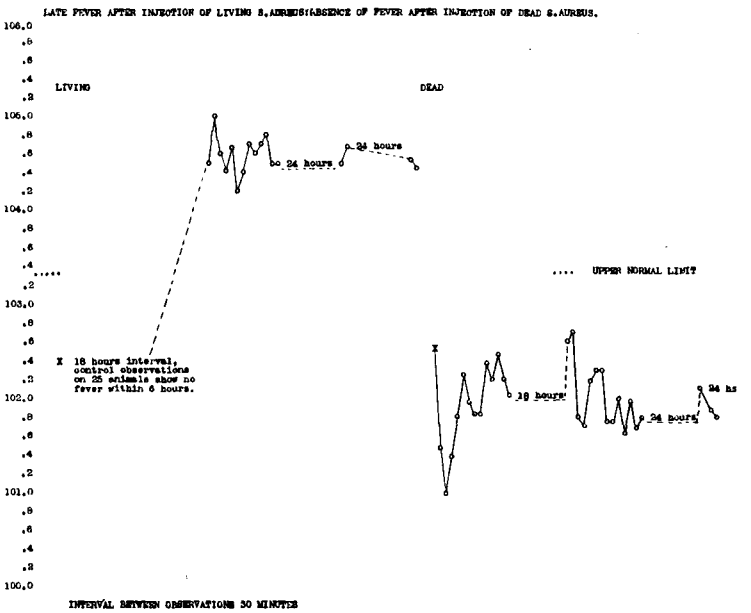
	NO. of animal	Weight	Broth in c.c		Water in c.c		Date of injection
A	49	2278	.13	1/17,523	nil		7.11.11
B	256	1913	.50	1/ 3,820	nil		25 3.12
C	332	1913	.97	1/ 1,972	nil		30. 4.12
D	334	1913	.97	1/ 1,972	nil		30. 4.12
E	330	2026	1.03	1/ 1,968	20.20	1/100	30.4.12
F	331	1913	.97	1/ 1,972	19.10	1/100	30.4.12

Interval between observations 30 minutes.

Chart 26.

applied. Moreover the morphological aspects of the stained organism itself are microscopically in no way affected by what appears to be a process of oxidation, although the degree of deglutination effected is often striking. Finally, if the organisms in as dry a condition as it is possible to get them without desiccation are submitted to prolonged heating in the water bath at boiling point the pyrogen becomes to a large extent inactive when the residue is again taken up in water. This residue loses the original colour of the medium whilst the staining properties and the integrity of the organisms as seen by the microscope are in no way impaired.

If we now turn to group B a marked contrast is seen. If for example intravenous injections of vaccines of *S. aureus* are given after destruction by boiling no fever whatever follows the injection, either immediately or on any subsequent day up to a fortnight—the limit of our observations—whatever the number of organisms injected. This was shown in Charts 17 and 18. If after a small injection of a few million organisms a day, a week, or a fortnight be allowed to elapse, and if then an injection of several thousand million organisms be given, no fever ensues. If these living organisms be injected intravenously there



NO of animal	Weight	Quantity of culture	Heated	Date of culture and source	Date of injection
567	2590	1/10 c.c of emulsion 1 1.0 c.c water	no	17.6.12 Agar	18.6.12
566	2419	deposit of 2.0 c.c 16 hrs.broth culture	yes	17.6.12 Broth	18.6.12

Chart 27.

is again no immediate fever, but on the next day fever begins, and it merges into a definite clinical fever of a well-marked type, which continues for several days. This is seen in Chart 27.

It may be well to sum up the different behaviour as regards fever production between organisms in group A, as represented by *B. typhosus*, and organisms in group B, as represented by *Staphylococcus aureus*.

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The first produces, when injected alive or dead, an immediate fugitive fever, whilst the second produces when injected alive or dead no immediate fever. On the other hand, dead organisms of the first or second group produce no late fever, whilst injections of living *Staphylococcus aureus* produces well-marked fever of a continuous type, after a very definite latent period. The immediate fugitive fever with no latent period induced by injection of living or dead organisms in the first group is moreover caused by a heat stable substance indestructible by prolonged boiling or even by submission to a temperature of 120° C. in the autoclave. In this respect it behaves in an exactly similar manner to the pyrogenetic body in ordinary water or saline proved again and again often to contain no organisms capable of cultivation. On the other hand living organisms in group B are heat labile in respect of their pyrogenetic properties and produce a continuous fever after a well-marked latent period, whilst the dead organisms produce no fever immediate or remote, whether on first injection or on subsequent injection.

On the whole the evidence suggests, but does not absolutely prove, that the immediate fever which follows the injection of living or dead organisms in group A is due to introduction of an adventitious heat stable pyrogen derived from the medium, and is not due to pyrogen extracted from the organisms. If this be the case to the five fallacies we have described there must be added a sixth—the fallacy of attributing fever caused by the injection of extraneous products to derivatives of bacterial cells. The belief in the ability of dead organisms to produce fever in disease is to a large extent based on the immediate fever that follows their injection into animals, or that may follow their injection into man. Recognition of this source of fallacy, if it be a fallacy, is therefore vital.

On the other hand the fact, if it be a fact, that the immediate fever caused by organisms in group A is not a bacterial fever, is no evidence that dead organisms killed in the body are not capable of producing fever.

It is conceivable that organisms after growth on artificial media may be incapable when injected dead, of producing true fever in the body and may yet acquire this property after training and growth on the natural medium offered by an infected host.

In our search therefore for the source of the pyrogen of infective disease we have had to discard the use of organisms grown on artificial media, and to examine instead the pyrogenetic properties of organisms

grown on natural media. This we have done in two ways. In a series of experiments by the first method we exposed defibrinated blood freshly drawn from the rabbit to aerial infection for 30 minutes, and then submitted the blood in a sealed vessel to a temperature of 37° C. for a period of 24 hours. This blood when injected unheated in small quantities produced a fever intermediate in point of time of appearance between the early and late fevers described. There is, in short, in these experiments no immediate fever, but a late fever with the latent period very much shortened. Control observations with uninoculated blood showed complete absence of fever immediate or remote. Cultures from the fever producing blood showed abundant growth of chromogenic cocci. Injection of the heated deposit from this blood, shown by the microscope to have a high bacterial content, produced no fever. We have therefore here evidence that the living organisms after training in fresh defibrinated blood can produce fever of a different type to that produced by organisms grown on agar or on peptone broth. In experiments using the second method we injected into healthy rabbits *S. aureus* obtained from aerial infection of agar. The organisms were then injected into rabbits and were recovered in 24 hours by centrifuging the heart's blood. In all cases injection of the killed organisms obtained from the heart-blood, produced no fever, whilst injection of sufficient quantities of the living organism recovered in the same way never failed to do so. Although we have several charts showing the points mentioned we prefer to reserve them till this branch of the work is completed. It requires in fact much further study which will be reported on in due course. Enough, however, has been said to show that it opens up a promising line of enquiry.

In conclusion we wish to glance at the practical bearing of our experiments on vaccine injections in man. If, as we believe, we have produced evidence that the immediate fever set up by injection of organisms in group A is due to the injection of highly toxic bodies of non-bacterial origin their effect for good or evil cannot be ignored. If it is an advantage in the treatment of an established infection to occasion fever by the injection of impure vaccines, standardization of the pyrogenetic content of a vaccine will in the future be no less necessary than that of its bacterial content. We have already shown that vaccines made from the same variety of organism in group A will vary from one another in respect of pyrogen unless precautions are taken to ensure the same period of growth on the same kind of medium. It is impossible to forget that the organisms for which the greatest measure of success

in vaccine treatment has been claimed are those in which the content of extraneous pyrogen is highest—the acne bacillus, *B. typhosus*, *B. coli*, the *Gonococcus* and *Micrococcus catarrhalis*. On the other hand, though in the absence of further evidence it would be absurd to press the point, the vaccines which have so far given the poorest results in the production of active immunity in man are just those in which we have found no adventitious pyrogen—*Pneumococcus*, *Streptococcus*, *Staphylococcus aureus* and diphtheria. If on the other hand the presence of non-bacterial pyrogen is no merit in a vaccine the whole system of preparation of vaccines will have to be recast and steps taken to ensure destruction of pyrogen that will not involve impairment of any specific antigenic values the purified vaccines may possess. That these adventitious fever bodies have no effect on opsonic readings or other immunity reactions will also have to be shown if the hope of placing therapeutic inoculation on a sound experimental basis is to be realised. Incidentally will be noted the bearing of these experiments on the question of the relative merits of living and dead organisms as effective immunizing agents.

Finally, we feel justified in insisting that no injection experiments dealing with so-called protein fever, endotoxin fever, anaphylactic fever or bacterial fever can stand unless based on clear recognition of the numerous sources of fallacy we have named.

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